SALT REQUIREMENTS AND SPACE ORIENTATION OF THE LITTORAL ISOPOD LIGIA IN BERMUDA[1]

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Introduction

The important problem of the invasion of terrestrial or fresh-water habitats by marine organisms has received considerable attention in recent years (cf. Schlieper, 1929; Pearse, 1929; Pantin, 1931). The Isopoda extend from great depths of the ocean to terrestrial and fresh-water environments and should afford especially interesting material for these investigations. Tait (1916) in particular has studied the longevity of the littoral form, *Ligia oceanica*, in dilutions of sea water. The structure of *Ligia* has been described in detail in the monograph of Hewitt (1907).

The present paper deals chiefly with the effect of changes in the salt content of sea water on *Ligia baudiniana* Milne-Edwards, the common isopod in Bermuda.

Habits

Ligia baudiniana may be found in great numbers on the rocky shore (Verrill, 1903), especially in the intertidal zone at low tide. *L. oceanica* (Tait, 1916) sometimes remains covered with the tide but *L. baudiniana* retreats as the water advances. When isolated on stones in tidal pools, the isopods will run from one side of the rock to the other as if seeking a dry pathway to the shore. Occasionally I have observed them leaping from one stone to another to avoid the water. However, they are dependent on the sea water to keep the surface of the gills moist and we have never found specimens more than seventy feet from the sea; they appear in great numbers on rocks and walls several feet from the sea on rainy days. If placed in a terrarium containing a small pool of sea water, they will crawl to the edge of the water, turn around, and dip the ends of the uropodal spines in the water. By bringing the spines close together and altering the distance between the spines in a slow rhythm, the isopod moistens the gills with the water which rises between the spines by capillarity (Fig. 1). In this way a large drop of water may form on the gills.

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These spines, which are usually long, are also used as swimming fins and feelers as in other species of *Ligia*.

It is probable that *Ligia* enters the sea to release the young from the brood pouch for this occurred only in submerged specimens.

An examination of the gut contents revealed vegetable debris and unicellular algae. According to Hewitt (1907) *L. oceanica* feeds largely on decaying vegetable matter. *L. exotica* is described as omnivorous by Pearse (1931). Although Pearse (1929) is undoubtedly correct in pointing out that food supply alone is probably not the most important factor in determining the habitat of littoral forms, yet it is of interest to note that *Ligia baudiniana* subsists largely on the green coating of rocks in the intertidal zone.

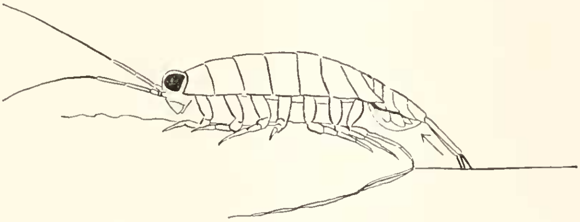

FIG. 1. *Ligia baudiniana* wetting the gills by the capillary action of the uropodal spines which are dipping into the sea.

CONCENTRATION EFFECTS

As a basis for comparison for subsequent experiments, the longevity of the isopods was first determined in sea water, air, and in fresh water. Individual specimens, carefully collected to avoid injury, were placed in finger bowls containing 100 cc. of water or solution. The average duration of life was only four hours in distilled water, seven and one-half hours in fresh water and thirty-four hours in sea water (Table I). The maximum longevity is also given in the tables. Sea water, changed every twenty-four hours, gave an average duration of life of fifty-eight hours, and in running aerated sea water the average longevity was one hundred and ninety-two hours, but the maximum was recorded for unchanged sea water. The large surface exposed to the air in the finger bowls permitted considerable diffusion of O_2 and CO_2 as is indicated by the maximum of twelve and one-half days in unchanged sea water. In dry glass dishes the average duration (and the maximum) was eleven hours, but in bowls containing damp sand the isopods lived for very long periods (fifteen days). It is clear that moist air is a far more favorable medium than sea water. *Ligia* dies in about one hour in dry air in the sun at 30–37° C. In sea water the isopods survive overnight at a temperature of 5° C., but the gills cease beating at 15° C.

The life of *Ligia* in sea water is markedly curtailed by dilution below 50 per cent or by doubling the concentration of salts (Table II). In dilute sea water there is a slight increase in the frequency of gill movements. The brief existence of the isopod in distilled water is not due to the decreased osmotic pressure, for the addition of glycerine has little beneficial effect (Table II). On the other hand, the death of *Ligia* in concentrated sea water appears to be due largely to osmotic factors as will be seen in the records of rapid death for sea water in which the osmotic pressure was increased by glycerine.

TABLE I

Longevity of Ligia in Air and in Water

Medium	Average Duration of Life	Maximum	No. of Specimens Tested
	hours		
Distilled water	4	5	21
Fresh water	7½	8	6
Sea water	34	297	93
Sea water (changed daily)	58	198	20
Running sea water	83	192	26
Air	11	12	8
Air over damp sand	360	625	31

TABLE II

Concentration Effects on Ligia

Medium	Average Longevity	Maximum	No. of Specimens
	hours		
25% sea water	6	10	6
50% sea water	20	70	7
75% sea water	51	172	8
200% sea water	3	7	5
250% sea water	1	1	4
400% sea water	1	2	3
M/2 glycerine	7	10	5
M glycerine	5	12	13
M/4 glycerine in sea water	11	24	8
M/2 glycerine in sea water	9	19	6
M glycerine in sea water	4	5	6
2 M glycerine in sea water	2	2	3

SPECIFIC ION EFFECTS

Of solutions containing a single salt isosmotic with Bermuda sea water (5/8 M), the isopods lived longest in NaCl, eight hours, and $CaCl_2$, seven hours; while the average duration of life in $MgCl_2$ and KCl was only four and one-half hours respectively (Table III). KCl exerted an immediate paralyzing effect on the gills which normally began to vibrate as soon as the animal was immersed in any of the solutions mentioned in this paper except KCl. The frequency of gill movements was taken at intervals in all solutions, but no vibrations were ever observed in KCl although the animals appeared quite normal for the first half hour. The average time for ten beats was three and one-fifth seconds in sea water (27°); and approaching death was indicated when this increased to five seconds. Immature specimens (12–15 mm.) had a faster rate, two and one-tenth seconds, and were not used; in addition it was found that immature specimens showed greater resistance to all solutions tested.

TABLE III

Specific Ion Effects on Ligia

Solution	Average Length of Life	Maximum	No. of Specimens
	hours		
5/8 M NaCl	8	14	12
5/8 M $CaCl_2$	7	13	19
5/8 M $MgCl_2$	3	5	7
2.5/8 M $MgSO_4$	4	4	10
1 M $MgSO_4$	3	4	8
5/8 M KCl	$1\frac{1}{2}$	3	10

IONIC ANTAGONISM

Combinations of two ions were tried in various proportions, but no satisfactory solution was found. No specimens lived for more than a very few hours in binary mixtures but some antagonism seemed evident between Na and Ca. In solutions containing Mg or K the longevity seemed to be controlled by the amount of the most toxic ion present. In artificial sea water (*i.e.*, 100 NaCl, 11.6 $MgSO_4$, 2.2 KCl and 2.5 $CaCl_2$ in 5/8 M conc.) the duration, forty hours, compared very favorably with natural sea water. In artificial sea water containing no magnesium, the same average duration of life was exhibited (Table IV). If the KCl was omitted, the isopods lived for twenty hours; in the absence of Ca, fourteen hours; and they died within two hours in artificial sea water containing no Na.

The next step was to determine how long *Ligia* would survive if the concentration of individual ions were increased in sea water. In the case of Na, Ca and Mg concentrations not exceeding M/8 made up in sea water (*i.e.*, one-eighth of the molecular weight added to a liter of sea water) did not exert an appreciable toxic effect but KCl showed a limiting concentration of M/10 in sea water (Table V).

TABLE IV

Longevity of Ligia in Antagonistic Solutions

Solution	Average Length of Life	Maximum	No. of Specimens
	hours		
Artificial sea water	40	123	8
Same without Na	2	2	5
Same without Ca	14	23	10
Same without K	20	28	9
Same without Mg	41	120	22

TABLE V

Effect of Increasing Concentration of Ions in Sea Water

Solution	Average Longevity	Maximum	Number of Specimens
	hours		
5/8 M NaCl made in sea water	$9\frac{1}{2}$	36	10
1/4 M NaCl made in sea water	11	27	5
1/6 M NaCl made in sea water	6	11	8
1/8 M NaCl made in sea water	57	244	5
2.5/8 M $CaCl_2$ made in sea water	$1\frac{1}{2}$	$2\frac{1}{2}$	9
1/8 M $CaCl_2$ made in sea water	88	248	8
2.5/8 M $MgCl_2$ made in sea water	3	6	5
1/8 M $MgSO_4$ made in sea water	91	258	5
5/8 M KCl made in sea water	2	3	13
1/4 M KCl made in sea water	$3\frac{1}{2}$	5	9
1/6 M KCl made in sea water	5	5	9
1/8 M KCl made in sea water	$6\frac{1}{2}$	16	9
1/10 M KCl made in sea water	31	201	14

The gills were completely inhibited in 5/8 M KCl in sea water but exhibited the usual rhythm in M/4 KCl in sea water. In all these solutions in which salts were added to sea water it is probable that the increased osmotic pressure was significant judging from the short life of isopods in sea water containing glycerine (Table II).

SPACE ORIENTATION

Ligia baudiniana inhabits a very restricted zone along the shore line, and a number of experiments were performed to discover what tropisms or other reactions restricted the distribution of the isopod. One may mention first the inability to survive in sea water or in dry air and the presence of food (unicellular algæ) on the intertidal rocks. These facts, however, do not explain the curious ability of the animal to orient towards the sea when released a short distance from the shore. It was noted that the isopods appeared to be reacting to the inclination of the land sloping gradually to the sea and it was found that under controlled conditions (in a photographic dark room under dim red illumination) pronounced geotropic orientation was exhibited.

Table VI

Orientation of Ligia on Slopes near the Sea

Date	Temp.	Direction of Slope	Inclination	Number Released	Direction of Creeping
	° *C.*		*degrees*		
July 10	27	Towards sea	30	6	5 down
11	27	Towards sea	40	2	down
11	27	Away from sea	40	2	down
Aug. 1	26	Towards sea	40	9	7 down 1 up 1 went up but turned
12	27	Away from sea	30	6	5 down
14	25	Towards sea	40	5	down
14	25	Right angles to sea	60	4	3 down
14	25	Away from sea	20	4	3 down 1 up
14	25	Away from sea	45	4	2 straight down 3 down obliquely
24	28.6	Away from sea	60	4	3 down
25	27	Towards sea	50	3	down
25	27	Away from sea	20	5	4 down
25	27	Away from sea	30	10	4 down 6 up

To test this hypothesis, specimens were released at various distances from the sea on ground (sand, grass, or rocks) sloping towards and also away from the sea (Table VI). Of sixty-four isopods tested, fifty showed positive geotropic orientation and crawled downward regardless of the direction of the sea. They also showed a less pronounced tendency to crawl in the direction of open patches of sky and exhibited positive phototropism under controlled conditions.

When isopods are released in the sea at a distance not greater than eight feet from shore, they swim energetically to shore or crawl over the bottom directly to shore. In general, the animal crawls over the bottom and seldom exhibits the typical swimming movements unless in deep water. The cause of this orientation to shore in the sea is unknown; it is independent of currents or the direction of the sun. The animal is negatively rheotropic and will swim against currents in an aquarium even after the removal of antennæ and uropodal spines, but this has nothing to do with the shoreward orientation. Luther (1930) has shown recently that the antennules of crabs are receptors for rheotropism, but in *Ligia* the antennules are extremely small (cf. Hewitt, 1907), and it seems probable that currents in the water stimulate the legs.

Discussion

Ligia affords a striking example of a marine organism which is invading the land through the intertidal zone—an approach to terrestrial life which has not received sufficient attention in theoretical considerations of the evolution of land animals. In tropical islands, lacking fresh water, and where there is no pronounced temperature difference between sea and air, the intertidal zone becomes an important route for the invasion of the land (Pearse, 1929). *Ligia baudiniana* contrasts sharply with *L. oceanica* which, according to Tait (1916), may live over eighty days in sea water. However, like the beach crab *Ocypode*, *L. baudiniana* is dependent on sea water to keep the gills moist for aerial respiration. It also resembles the beach crab in its inability to withstand fresh water or diluted sea water, and is thus quite different from the marine *Gammarus*, which lives long periods in sea water diluted to .5 per cent (Adolph, 1925). The death of *Ligia* in distilled water and in glycerine solutions indicates that, like *Gammarus* (Loeb, 1903), loss of essential salts is more detrimental than osmotic disturbances in the medium. In spite of its terrestrial life, *Ligia baudiniana* is clearly a poikilosmotic form, although in diluted sea water the respiratory rate increases, which on Schlieper's (1929) theory might be due to osmotic work performed in partially resisting disturbance of the water and salt equilibrium.

The order of toxicity of single ions, K > Mg > Ca > Na, appears to be about the same for several Crustacea, *i.e.*, *Gammarus* (Adolph, 1925), *Artemia* (Martin and Wilbur, 1921), *Daphnia* (Berger, 1929), *Cambarus* (Helff, 1929), and is the reverse of the toxicity series for the egg of the sea urchin (Page, 1929). The rapid death in KCl is probably due in part to the lack of ventilation of the gills, which are unable to move in this solution. Zoond (1931) has shown that ventilation of the gill surface is of vital importance in Crustacea due to the extremely slow rate of diffusion of O_2 in water. The recent experiments of Bialaszewicz (1932) have demonstrated that the high toxicity of KCl for Crustacea is associated with its rapid disappearance from the blood into the tissues. According to Loeb (1903) Na, K, and Ca are necessary for the gill movements of *Gammarus*, but it resembles *Ligia* in certain other salt requirements, *i.e.*, there is no satisfactory binary mixture and Mg appears to be a dispensable ion.

The orientation of *Ligia* to the sea resembles that of young loggerhead turtles described by Parker (1922). The orientation of the isopod to the shore, when in the sea, appears to be an instance of definite orientation which is not governed by a simple type of tropistic response and is not unlike the orientation of *Onchidium* (Arey and Crozier, 1921), and of ants (Barnes, 1929). The fact that the orientation of *Ligia* in the sea is not exhibited at distances greater than ten feet from the shore precludes the possibility that the isopod reacts to the blue color of deeper water as in the loggerhead turtle (Hooker, 1910).

Summary

1. *Ligia baudiniana* moistens its gills by the capillary action of the uropodal spines.

2. The isopod survives best in damp air and is unable to live for long periods in sea water.

3. Changes in the concentration of sea water are detrimental, *i.e.*, *Ligia* is poikilosmotic.

4. The cations of sea water are toxic in the following order: K > Mg > Ca > Na. K exerts a specific paralyzing effect on the gill movements.

5. On land *Ligia* orients toward the sea. Positive geotropism appears to be the most important factor.

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